Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

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**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

***A projekt az Európai Unió támogatásával, az Európai Szociális Alap társfinanszírozásával valósul meg.

NEURAL INTERFACES AND PROSTHESES

(Neurális interfészek és protézisek)

LECTURE 2

BASICS OF ELECTRICAL STIMULATION

(Elektromos ingerlés alapjai)

RICHÁRD CSERCSA and GYÖRGY KARMOS

Investing in your future
New Hungary Development Plan

AIMS:

In this lecture, the student will become familiar with the necessary elements of electric stimulation.

One important element is an excitable nerve cell. The student will learn about the semi-permeable membrane, the ions producing the membrane potential, and the generation of the resting potential.

They will also learn about electric fields, currents, and the effect they have on neurons.

This lecture mentions the types of electrodes and stimulation techniques, introducing concepts essential in practice, such as non-polarizable electrodes, biphasic stimulation, chronaxy, and rheobase.

Duchenne, 1862 (commons.wikimedia.org)

Electric stimulation means a functional change in a nerve (muscle) cell due to electric current.

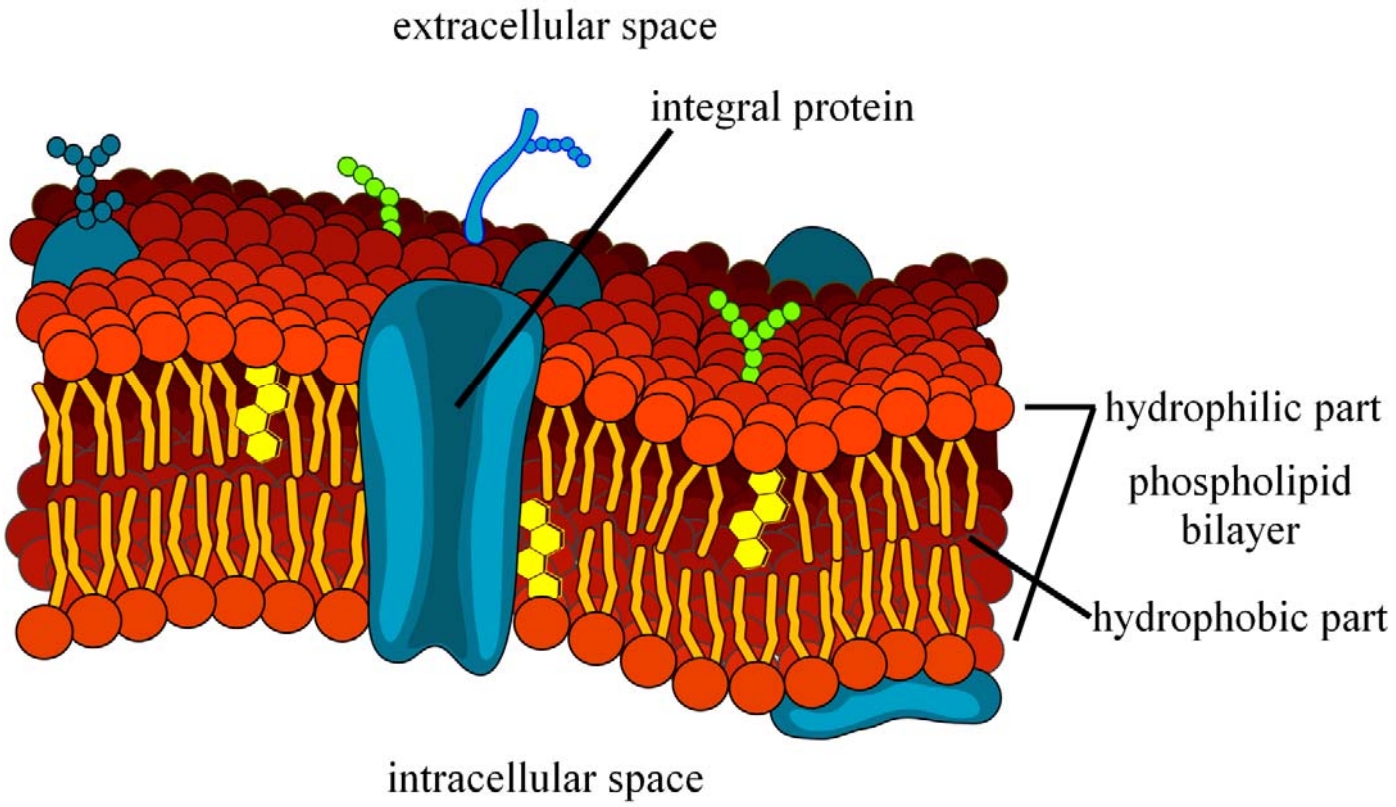
Needed:

- **Excitable cell**
- Electric current
- Stimulating electrode

Excitable cell:

- Cell membrane
- Membrane proteins
- Ion concentration difference between the two sides of the membrane

STRUCTURE OF NEURON MEMBRANE



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STRUCTURE OF NEURON MEMBRANE

The neuron membrane is a phospholipid bilayer that separates the intracellular and the extracellular fluids. Each phospholipid molecule consists of a hydrophilic and a hydrophobic part. Molecules are organized into layers such that hydrophobic parts are inside the membrane, and hydrophilic parts contact with the external/internal world. This structure makes the membrane not permeable for ions and charged molecules, thus a good dielectric.

Certain proteins may be embedded in the membrane. They can be peripheral, if they do not span through the whole membrane, or transmembrane, if they reach both sides of the membrane.

These proteins are called ion channels or transporters if they can transport ions from one side of the membrane to the other.

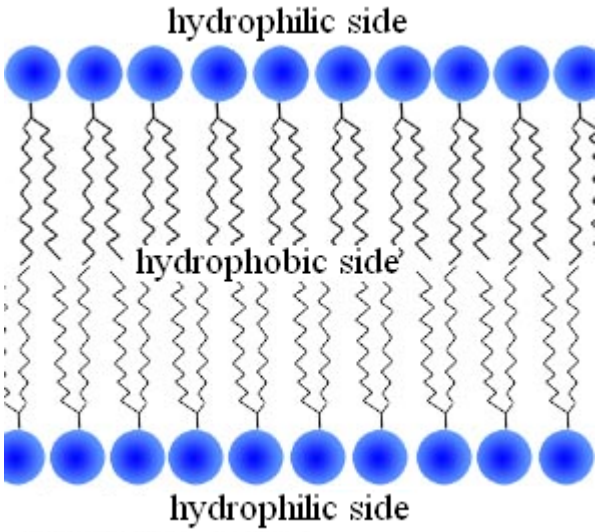
BUILDING BLOCKS OF NEURON MEMBRANE

phospholipid molecule

hydrophile

hydrophobe

phospholipd bilayer (membrane) in water



- good dielectric
- not permeable for ions, charged molecules
- not permeable for big molecules
- permeable for water and small, uncharged molecules
- molecules soluble in fat may dissolve in the membrane

(commons.wikimedia.org)

ION CHANNELS

Ions can be transported through the cell membrane passively, without energy investment, or actively, when energy is needed for the transport. The necessary energy comes from adenosine triphosphate (ATP) dephosphorylation.

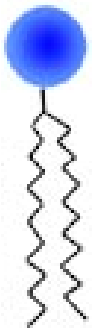
Ion channels can be closed, when they are in a conformation that they cannot transport ions, or open. Ion channels can be ligand gated or voltage gated, depending on the way they can become open. Ligand gated channels require certain molecules attached to them in order to open, while for voltage gated channels, a certain potential difference between the two sides of the membrane is necessary.

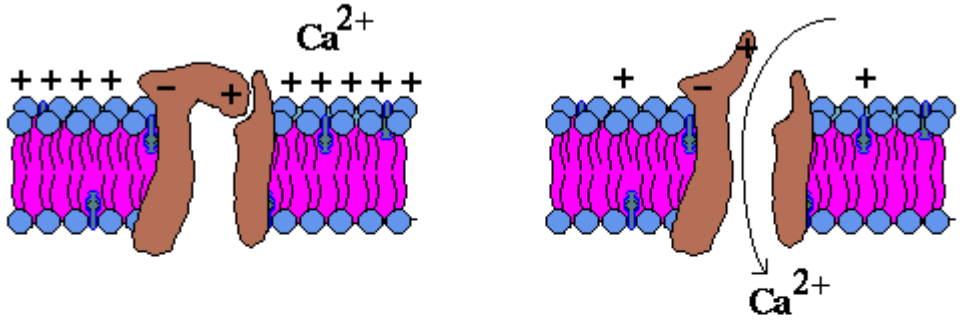
PROTEINS OF NEURON MEMBRANE

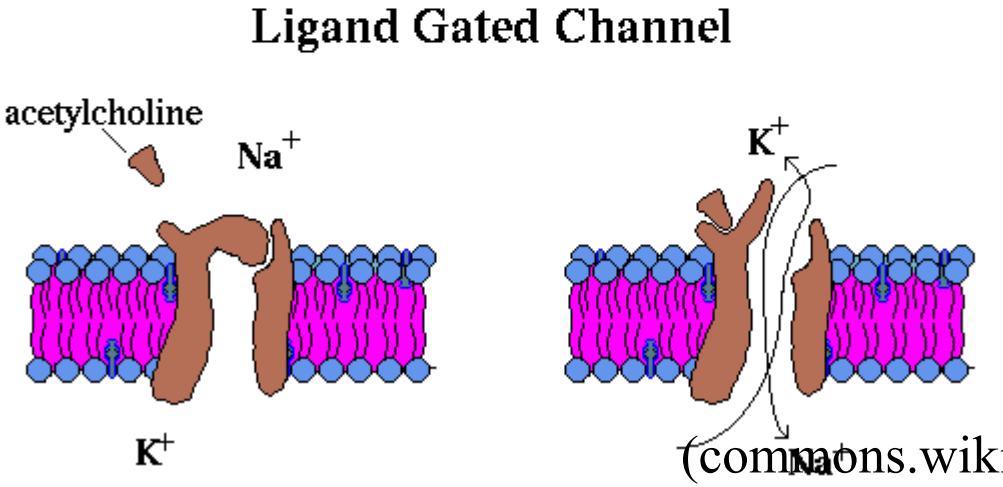
phospholipid molecule

hydrophile

hydrophobe







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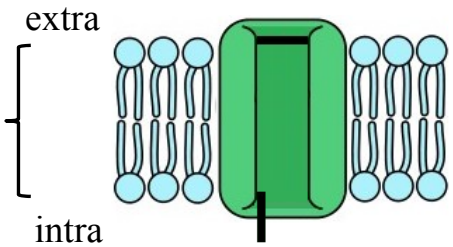
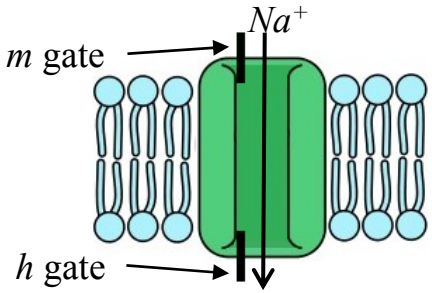
VOLTAGE-GATED NA$^+$ CHANNEL

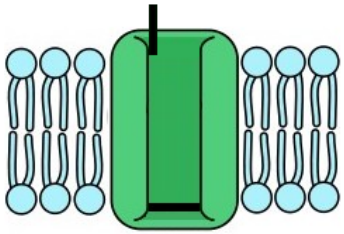
Plasma membrane



At rest
(V_m = -75 mV)



Immediately after
depolarization (V_m = -50 mV)



5 msec after
depolarization (V_m = -50 mV)

VOLTAGE-GATED NA^+ CHANNEL

At resting potential, the m gate of the channel is closed, therefore Na+ ions are not able to flow through the membrane.

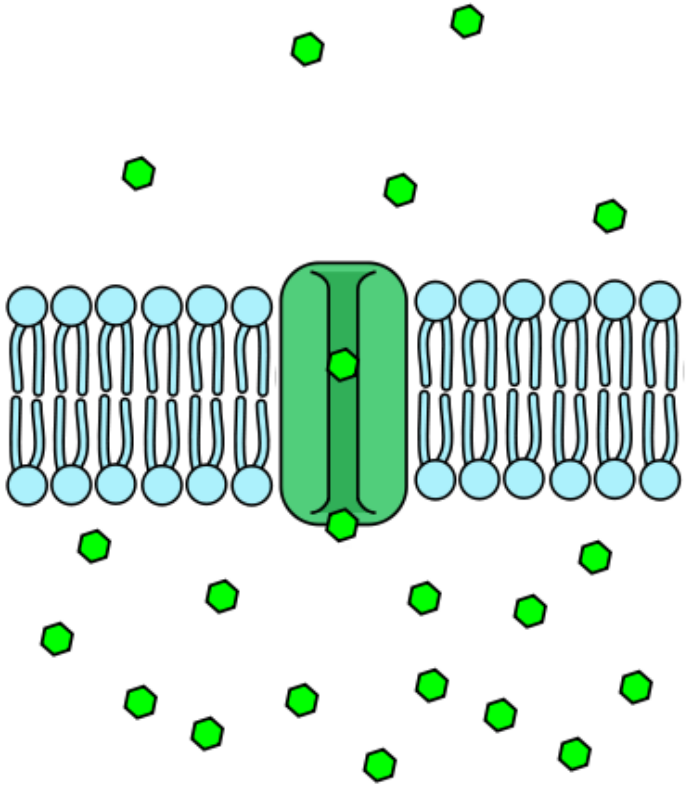
When the membrane is depolarized (the potential difference between the two sides of the membrane is smaller), both gates of the voltage-gated Na+ channel open, allowing the Na^+ ions to flow into the cell, further depolarizing the membrane.

Soon after the depolarization (~5 msec) the h gate of the channel closes, stopping the inward flow of Na^+.

DIFFUSION BETWEEN SPACES WITH DIFFERENT CONCENTRATION

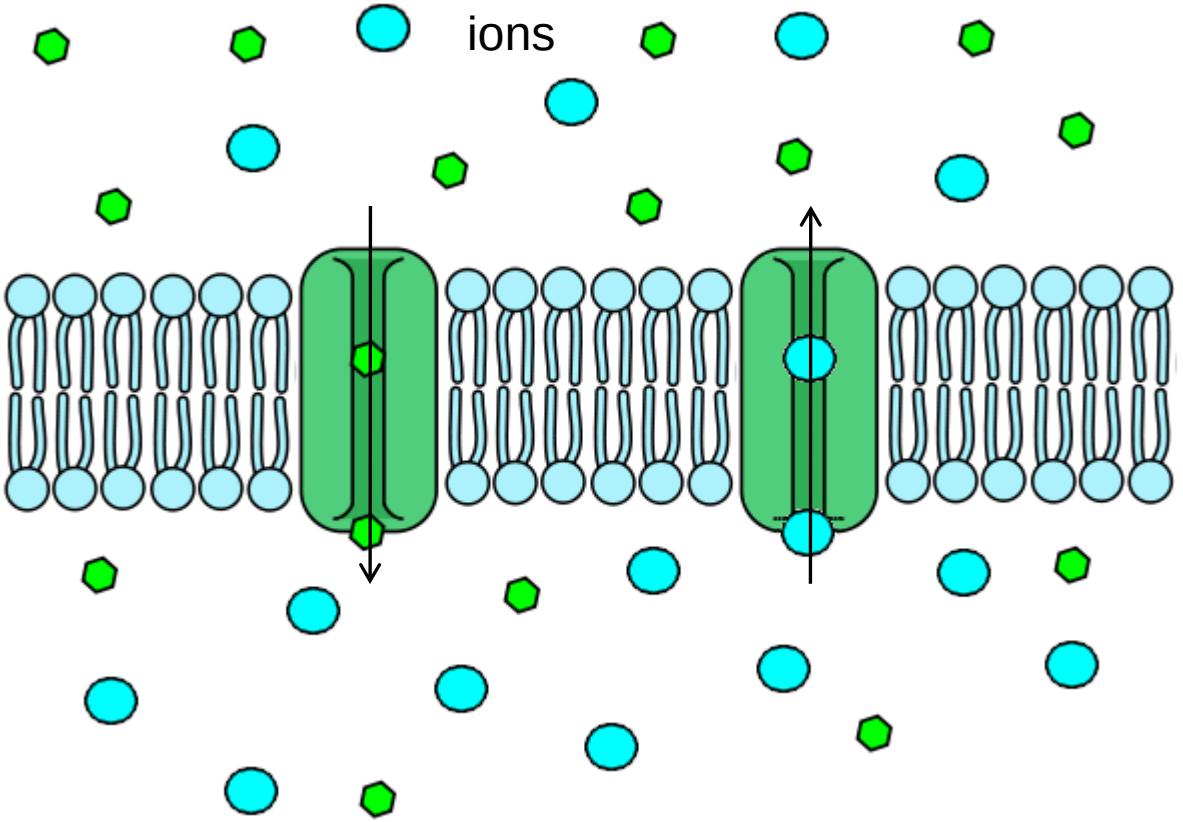
low
concentration

HIGH
CONCENTRATION



ION MOVEMENT IN ELECTRIC SPACE

positive potential



negative potential

ION MOVEMENT

The movement of ions through the membrane depends on

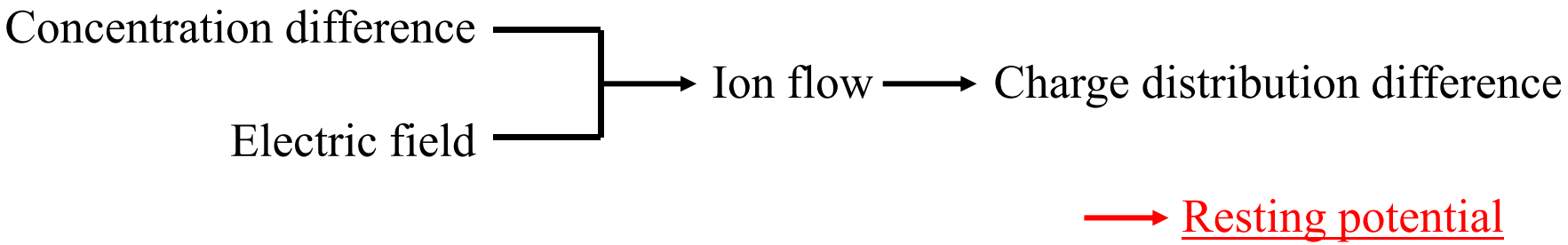
- the ion concentration gradient,
- electric charges.

If the concentration of a certain molecule is higher in one compartment than the other, they will diffuse to the compartment with lower concentration (diffusion force).

If the electric field is positive in one compartment, negative ions will tend to move there, while positive ions will move away and vice versa (electrostatic force).

Furthermore, ion movement is determined also by the type of open channels. Some channels are selective for ions (e.g. only cations, or only K^+ ions).

ION MOVEMENT



In a living cell $[K^+]_{intracell} > [K^+]_{extracell}$ and $[Na^+]_i < [Na^+]_e$

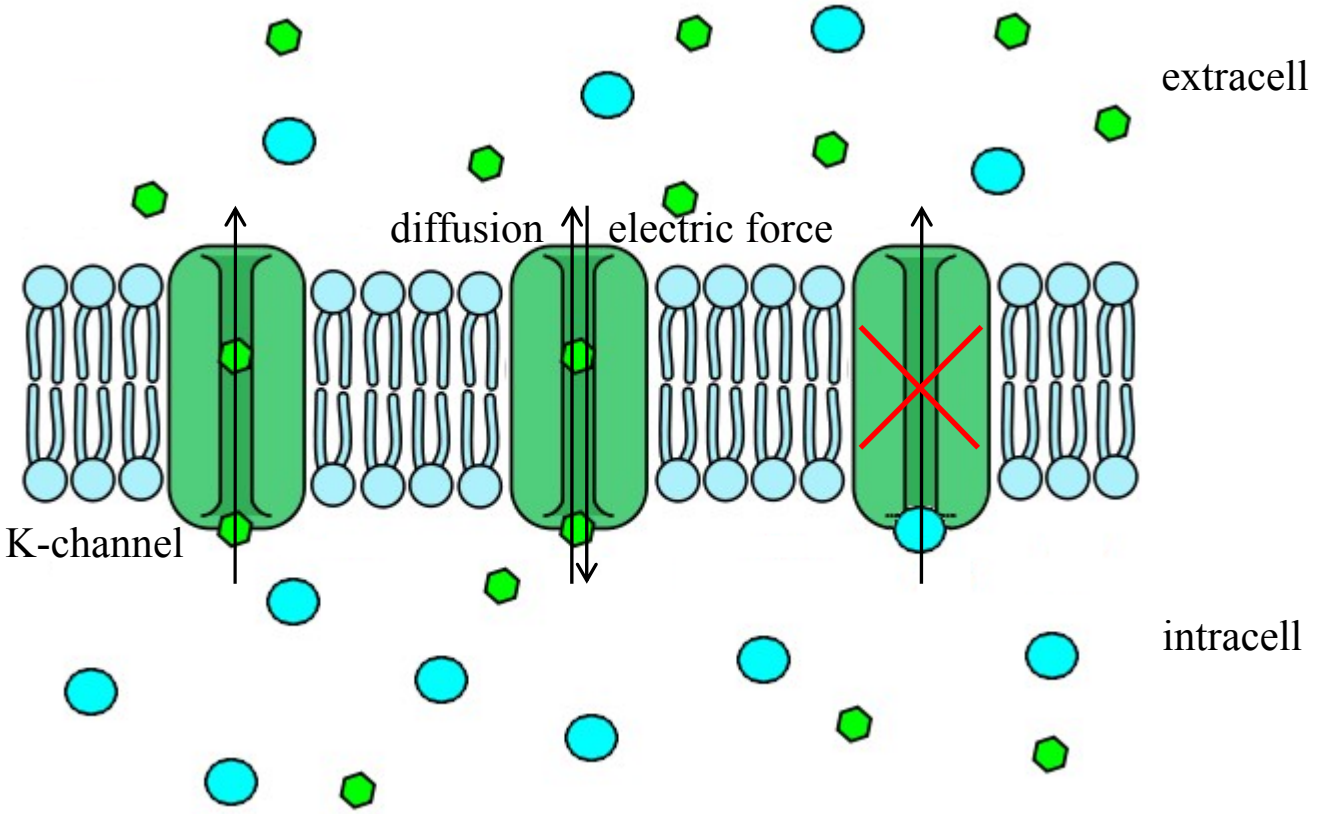
In a living cell at rest K^+ flows through the membrane much more easily than any other ions. If $p_K=1$ then $p_{Na}=0.1$.

Very few ions are transported, only small changes in concentration take place.

ION MOVEMENT THROUGH SELECTIVE CHANNEL

more positive charges
(+) POTENTIAL

less positive charges
(-) POTENTIAL



ION MOVEMENT

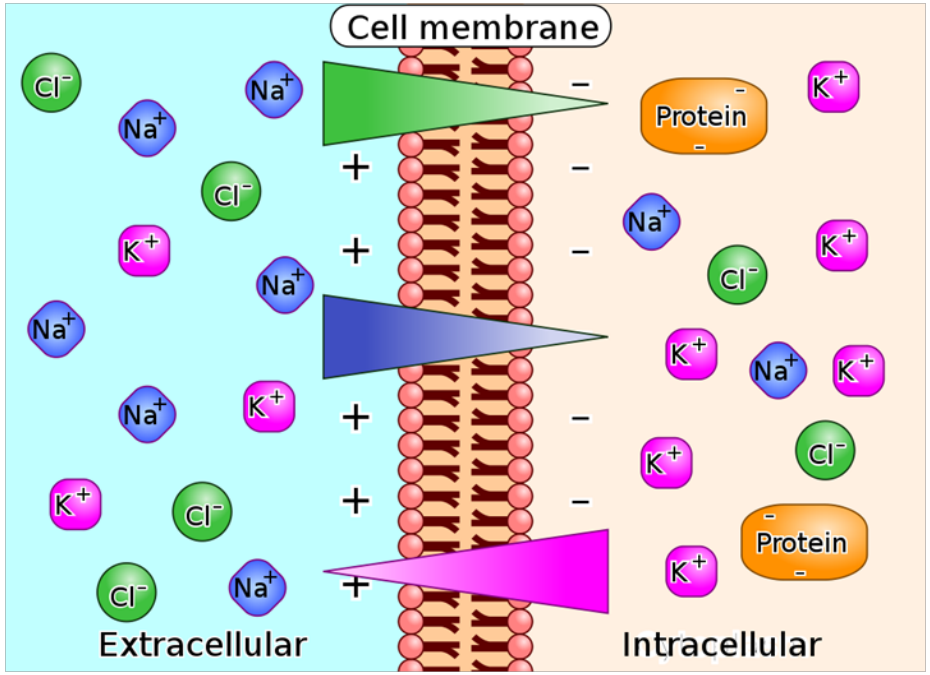
Electric field

$$j_{drift,p} = \frac{Z_p}{|Z_p|} \cdot C_p \cdot v_p = \frac{Z_p}{|Z_p|} \cdot C_p \cdot (u_p \cdot E)$$

drift flux j_{drift}
valence Z_p
concentration C_p
velocity v_p
mobility u_p

Diffusion

$$j_{diffusion} = -D \cdot \nabla C$$



diffusion flux $j_{diffusion}$
diffusivity D
concentration C

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Ion diffusion: $j_{diffusion} = -D \cdot \nabla C$

Electric field: $j_{drift,p} = \frac{Z_p}{|Z_p|} \cdot C_p \cdot v_p = \frac{Z_p}{|Z_p|} \cdot C_p \cdot (u_p \cdot E)$

$$j_p = j_{diffusion,p} + j_{drift,p} = -D_p \cdot \left(\nabla C_p + \frac{Z_p \cdot C_p \cdot F}{R \cdot T} \cdot \nabla V \right)$$

$$J_p = j_p \cdot (F \cdot Z_p) \qquad E = -\nabla V$$

$$J_p = -D_p \cdot (F \cdot Z_p) \cdot \left(\nabla C_p + \frac{Z_p \cdot C_p \cdot F}{R \cdot T} \cdot \nabla V \right)$$

$$D_p = \frac{u_p \cdot R \cdot T}{|Z_p| \cdot F}$$

No net current flow in equilibrium:

$$J_p = -D_p \cdot F \cdot Z_p \cdot \left(\nabla C_p + \frac{Z_p \cdot C_p \cdot F}{R \cdot T} \cdot \nabla V \right) = 0$$

R = gas constant = 8.314 J/K mole at 27 °C

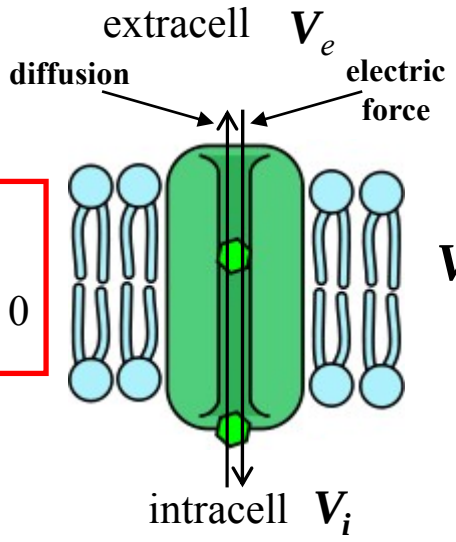
F = Farady = 96,487 coulombs/mole

Nernst equation

$$V_m = (V_i - V_e) = \left(\frac{R \cdot T}{Z_p \cdot F} \right) \cdot \ln \left(\frac{[C_p]_e}{[C_p]_i} \right)$$

NERNST EQUILIBRIUM

- ion selective membrane
- ion concentration difference
- mobile + ion (K, intracell)
- non mobile – ion (A, intracell)



Equilibrium

$I_{DIFF} + I_E = I_{net} = 0$

$V_m = V_k = V_i - V_e$

Vm: membrane potential

$c_{i,K} = 120\text{mmol/l}$
$c_{e,K} = 5\text{mmol/l}$
$V_k = 61 \log_{10}(24)$
$V_K = -84.19\text{mV}$

V_k= equilibrium potential

F = Faraday constant [9.649 × 104 C/mol]
T = absolute temperature [K]
R = gas constant [8.314 J/(mol·K)]
z_k = *valence*
$c_{i,k}$ = *intracell concentration*
$c_{o,k}$ = *extracell concentracion*

Nernst equation

$$V_k = -\frac{RT}{z_k F} \ln \frac{c_{i,k}}{c_{e,k}}$$

T = 273 + 37
z = 1

$$V_k = -61 \cdot \log_{10} \frac{c_{i,k}}{c_{e,k}} \text{[mV]}$$

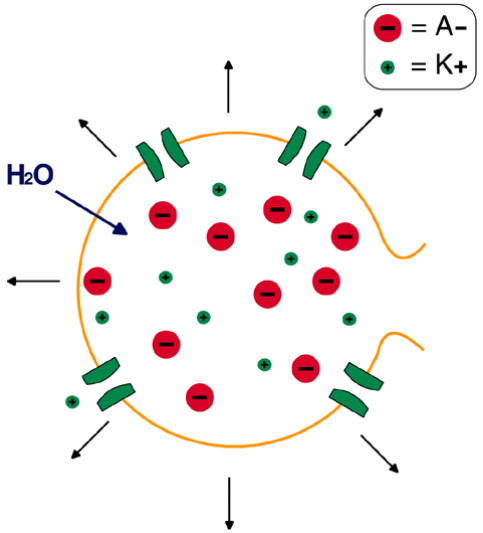
NERNST EQUILIBRIUM

For an excitable cell, a dielectric membrane and ion concentration difference on its two sides are essential.

Resting membrane potential is the membrane potential (the potential difference between the two sides of the membrane) when there is no net ion movement. It is an equilibrium state when the sum of diffusion and electrostatic forces is zero. It also means there is no net current flow through the membrane.

The Nernst equation gives the equilibrium potential of an ion, given its intra- and extracellular concentrations. This is the potential when there is no net movement of that ion. This value is proportional to the logarithm of the quotient of concentrations.

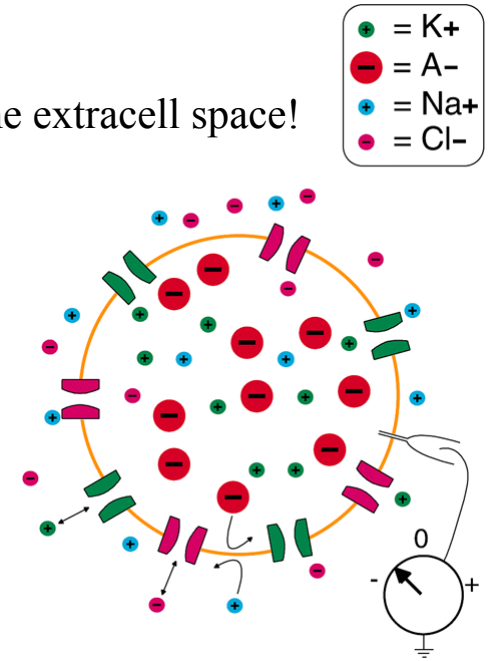
OSMOTIC CATASTROPHE



Due to high concentration of ions inside the cell, water would diffuse into the cell until it disrupts.

COMPENSATE FOR THE DIFFUSION OF WATER

NaCl in the extracell space!



Equilibrium

$I_{DIFF} + I_E = I_{net} = 0$

- **ion selective membrane**
- **ion concentration difference**
- **mobile + ion (K, intracell)**
- **non mobile – ion (A, intracell)**
- **mobile – ion (Cl, extracell)**
- **non mobile + ion (Na, extracell)**
- **compensated for water diffusion: $c_i = c_e$**
- **$c_{i,K} = c_{e,Cl}$ $c_{e,K} = c_{i,Cl}$**

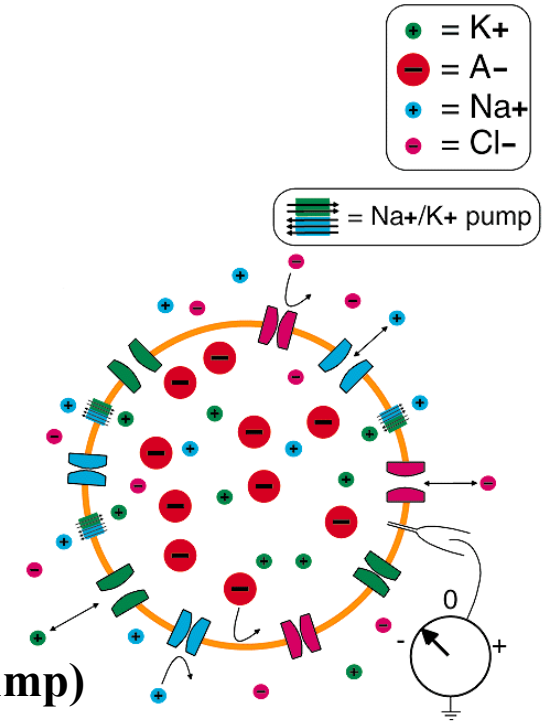
$$V_D = V_{Cl} = V_K = V_m = V_i - V_e$$

$$V_D = -61 \cdot \log_{10} \frac{c_{i,K} + c_{e,Cl}}{c_{e,K} + c_{i,Cl}} [mV]$$

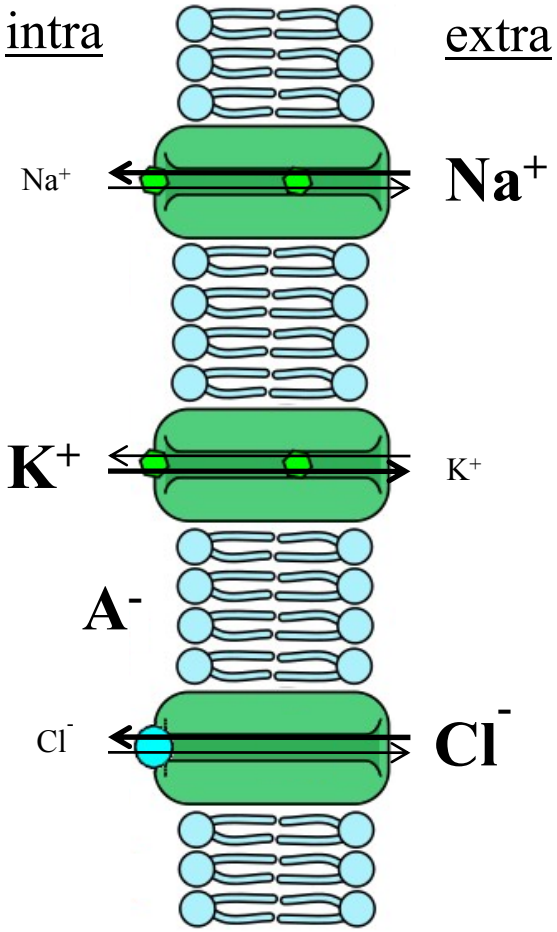
$$V_D = V_K = V_{Cl} = V_m = -84.19mV$$

REALISTIC CELL MODEL

- ion selective membrane
- ion concentration difference
- mobile + ion (K, intracell)
- non mobile – ion (A, intracell)
- mobile – ion (Cl, extracell)
- non mobile + ion (Na, extracell)
- compensated for water diffusion
- different permeability of ion channels
- active transport for maintaining ion gradient (Na/K pump)
- no equilibrium on ion channels (leak)



RESTING POTENTIAL



ion	intra [mmol/l]	extra [mmol/l]	V_k
Na^+	15	150	+61 mV
K^+	120	5	-84.19 mV
Cl^-	7.5	125	-74.53 mV

ion	permeability, P [cm/sec]
Na+	0.05×10^{-7}
K+	1×10^{-7}
Cl-	0.1×10^{-7}

Vr = -61.15 mV

$P_K > P_{Cl} > P_{Na}$

Goldman-Hodgkin-Katz equation

$$V_r = -61 \cdot \log_{10} \frac{P_K \cdot c_{i,K} + P_{Na} \cdot c_{i,Na} + P_{Cl} \cdot c_{e,Cl}}{P_K \cdot c_{e,K} + P_{Na} \cdot c_{e,Na} + P_{Cl} \cdot c_{i,Cl}} [mV]$$

Depolarization: Vm > Vr
Hyperpolarization: Vm < Vr

CHANGES IN MEMBRANE POTENTIAL

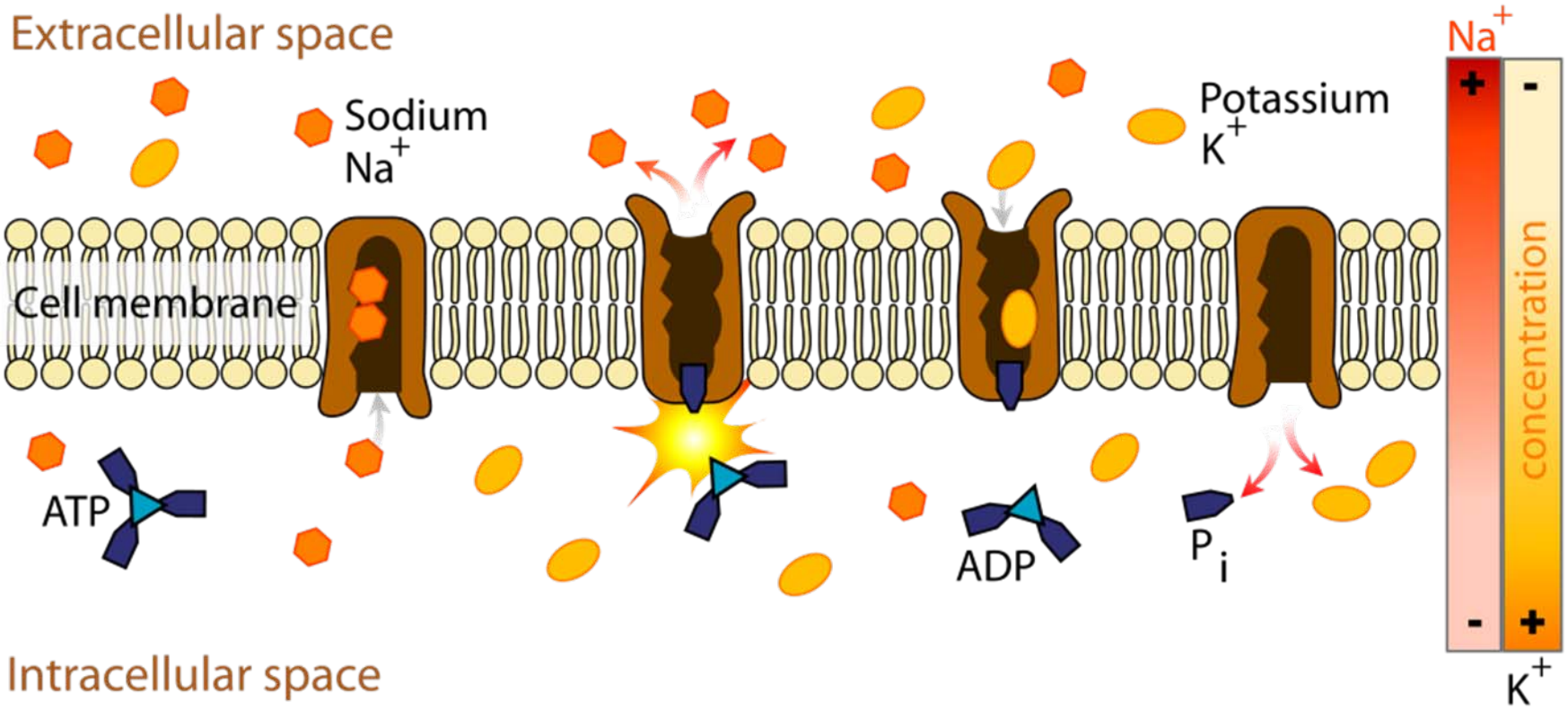
Depolarization: Vm > Vr
Hyperpolarization: Vm < Vr

P constant	Extracell concentration	Vm	Extracell concentration	Vm
Na^+	↑	D	↓	H
K^+	↑	D	↓	H
Cl^-	↑	H	↓	D

Concentration constant	P	Vm	P	Vm
Na^+	↑	D	↓	H
K^+	↑	H	↓	D
Cl^-	↑	H/D	↓	D/H

Channels wide open push the membrane potential to the equilibrium potential of given ion.

SODIUM-POTASSIUM PUMP



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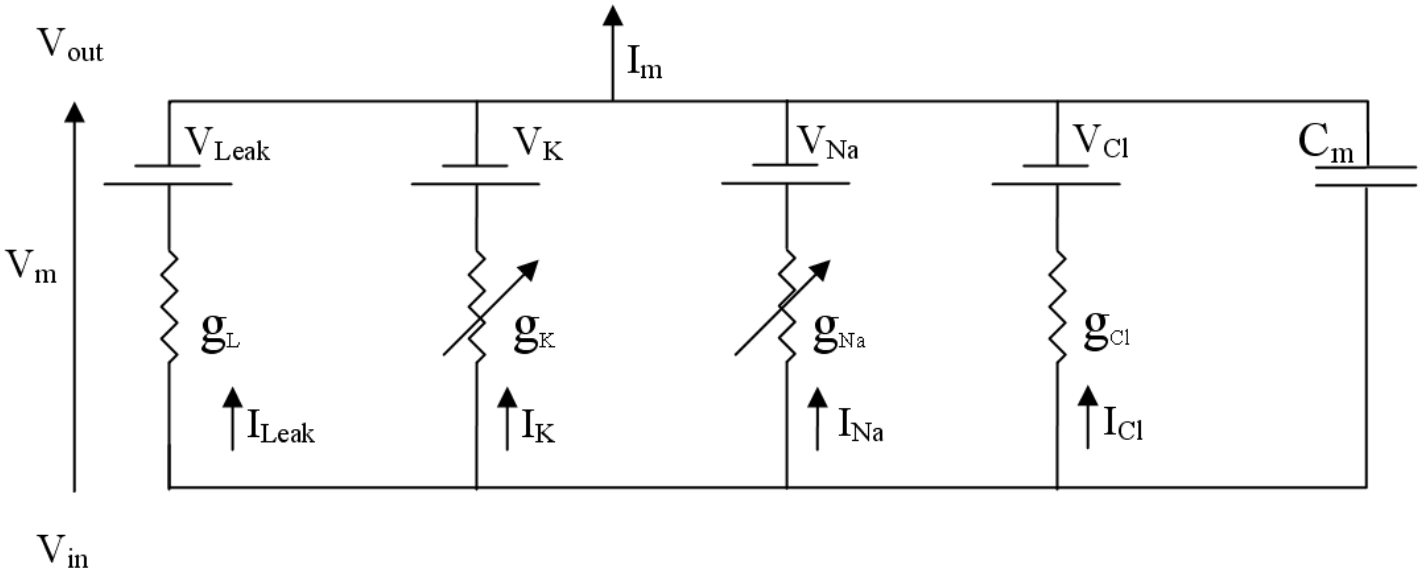
SODIUM-POTASSIUM PUMP

In order to maintain the physiological ion concentrations, ions transported through the membrane have to be transported back. This happens against their concentration gradient, thus requires energy.

This task is executed by an ion pump. The sodium-potassium pump takes three sodium ions back to the extracellular space and two potassium ions to the intracellular space. It uses ATP dephosphorylation to acquire the energy needed for the transport.

MEMBRANE EQUIVALENT CIRCUIT

extra



intra

R=U/I
G=1/R (conductance)

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Electrical stimulation means a functional change in a nerve (muscle) cell due to electric current.

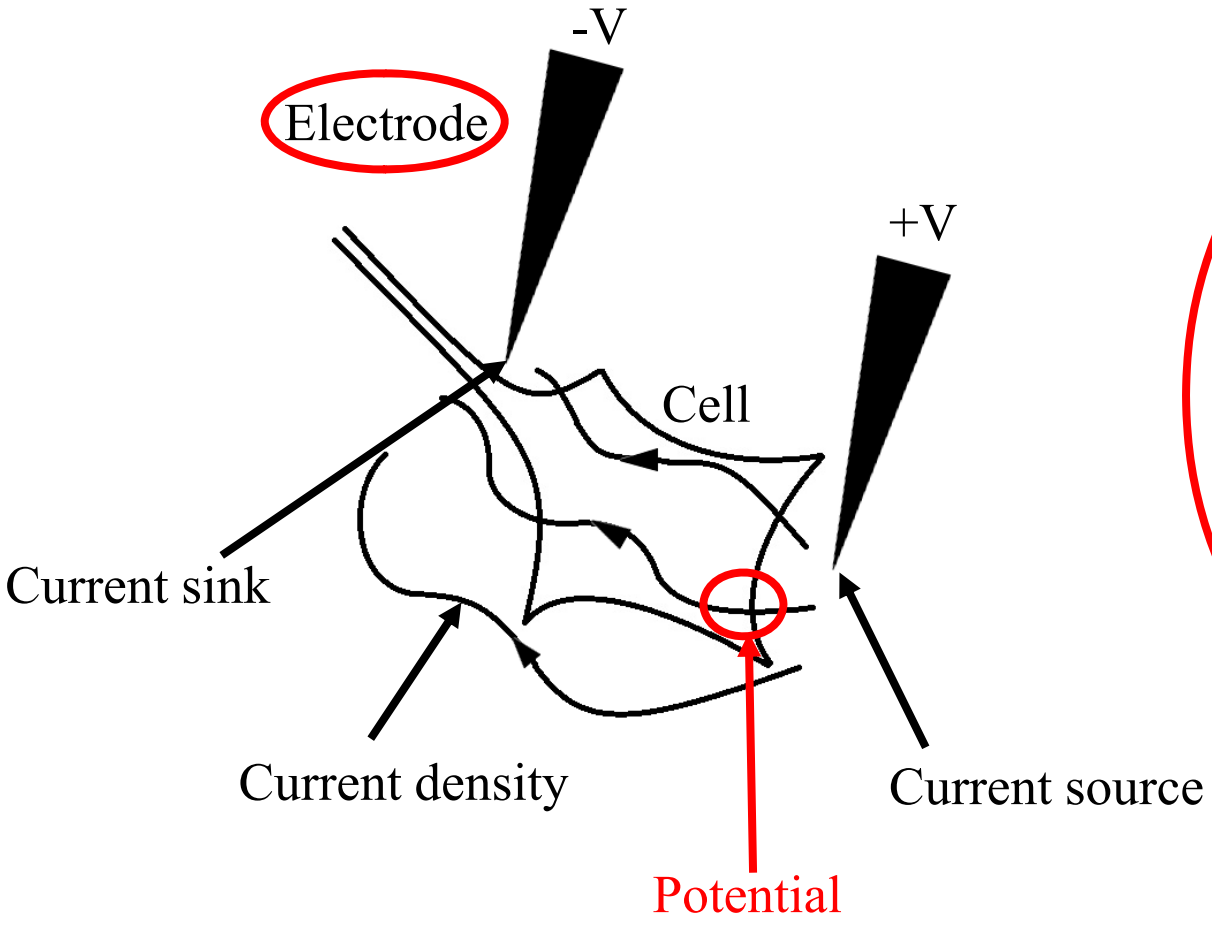
Needed:

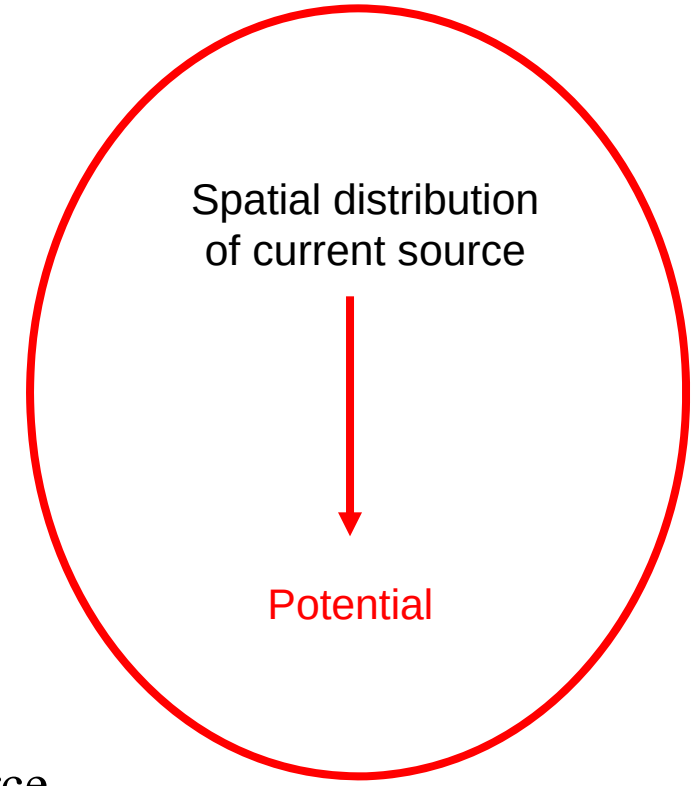
- Excitable cell
- **Electric current**
- Stimulating electrode

Electric current:

- Generate current space
- Define potential

STIMULATION WITH ELECTRIC CURRENT





FIELD THEORY: STATIONARY ELECTRIC FIELDS

Electric field: E

relation to potential: $E = -\nabla V$

force on charge: $F = q \cdot E$

Current density: J

relation to conductivity: $J = \sigma \cdot E$

current source density: $\nabla \cdot J = I_{source}$

(grad) $\nabla p = \frac{\partial p}{\partial x}\mathbf{i} + \frac{\partial p}{\partial y}\mathbf{j} + \frac{\partial p}{\partial z}\mathbf{k}$

(div) $\nabla \cdot \mathbf{A} = \frac{\partial A_x}{\partial x} + \frac{\partial A_y}{\partial y} + \frac{\partial A_z}{\partial z},$

(Laplace) $\nabla^2 f = \nabla \cdot (\nabla f) = \frac{\partial^2 f}{\partial x^2} + \frac{\partial^2 f}{\partial y^2} + \frac{\partial^2 f}{\partial z^2},$

Poisson equation: $\nabla \cdot J = \nabla \cdot [\sigma \cdot E] = \nabla \cdot [\sigma \cdot (-\nabla V)] = -\sigma \cdot \nabla^2 V = I_{source}$

Potential for given current source density:

$$V = \frac{1}{4 \cdot \pi \cdot \sigma} \int_{\upsilon} \frac{I_{source} \cdot d\upsilon}{r}$$

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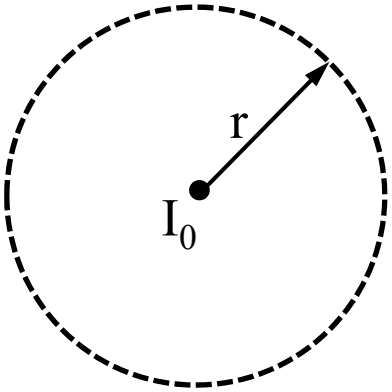
ELECTRIC FIELD OF MONOPOLE

$$\vec{J}(r) = \frac{I_0}{4 \cdot \pi \cdot r^2} \cdot \hat{r}$$



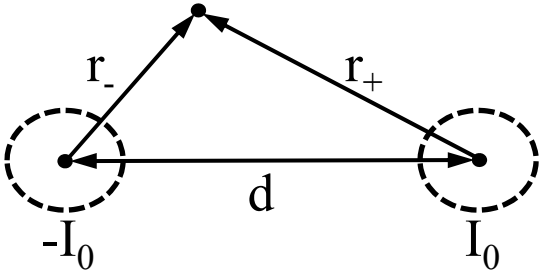
$$J = \sigma \cdot E \qquad E = -\nabla V$$

$$\nabla V = \frac{dV}{dr} = -\left(\frac{I_0}{4 \cdot \pi \cdot \sigma \cdot r^2} \right) \cdot \hat{r}$$

$$V = \frac{1}{4 \cdot \pi \cdot \sigma} \int_{\upsilon} \frac{I_{source} \cdot d\upsilon}{r}$$

$$V_{monopole} = \frac{I_0}{4 \cdot \pi \cdot \sigma \cdot r}$$

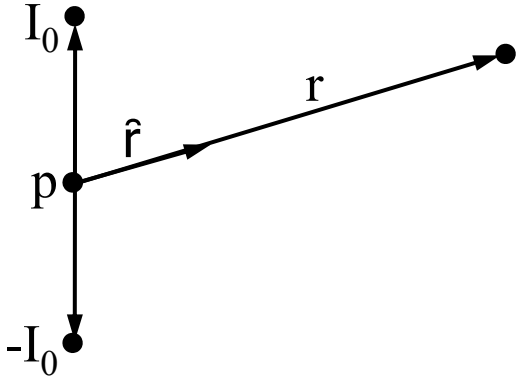
ELECTRIC FIELD OF DIPOLE



superposition:

$$V_{dipole} = \frac{I_0}{4 \cdot \pi \cdot \sigma \cdot r_+} + \frac{-I_0}{4 \cdot \pi \cdot \sigma \cdot r_-}$$

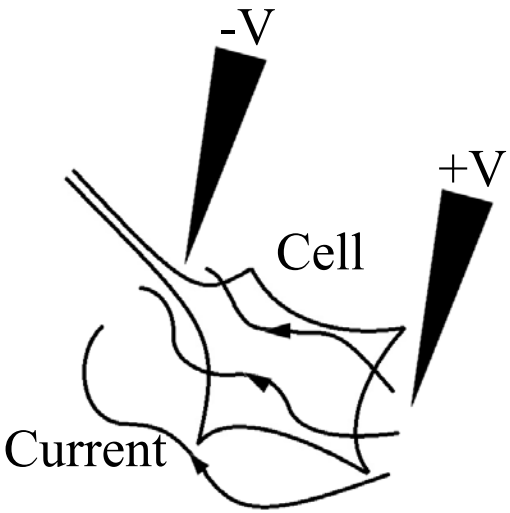
$$\vec{p} = I_0 \cdot \vec{d}$$ dipole moment

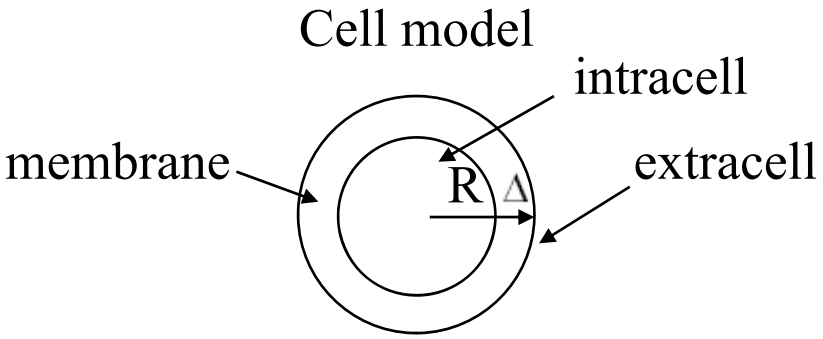


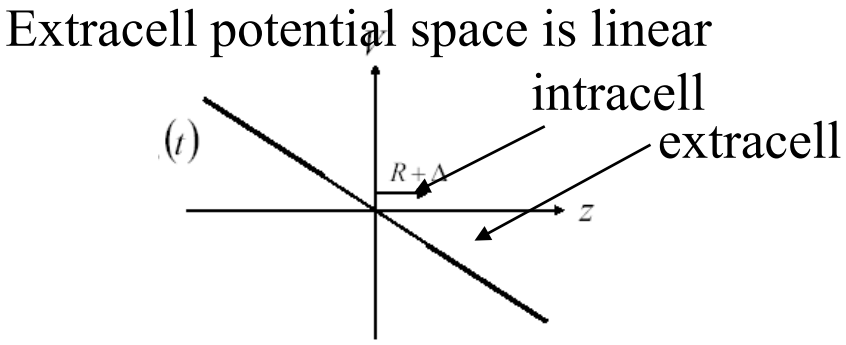
$$V_{dipole} = \frac{I_0 \cdot \left(\vec{d} \bullet \hat{r}\right)}{4 \cdot \pi \cdot \sigma \cdot r^2} = \frac{I_0 \cdot d \cdot \cos\theta}{4 \cdot \pi \cdot \sigma \cdot r^2}$$

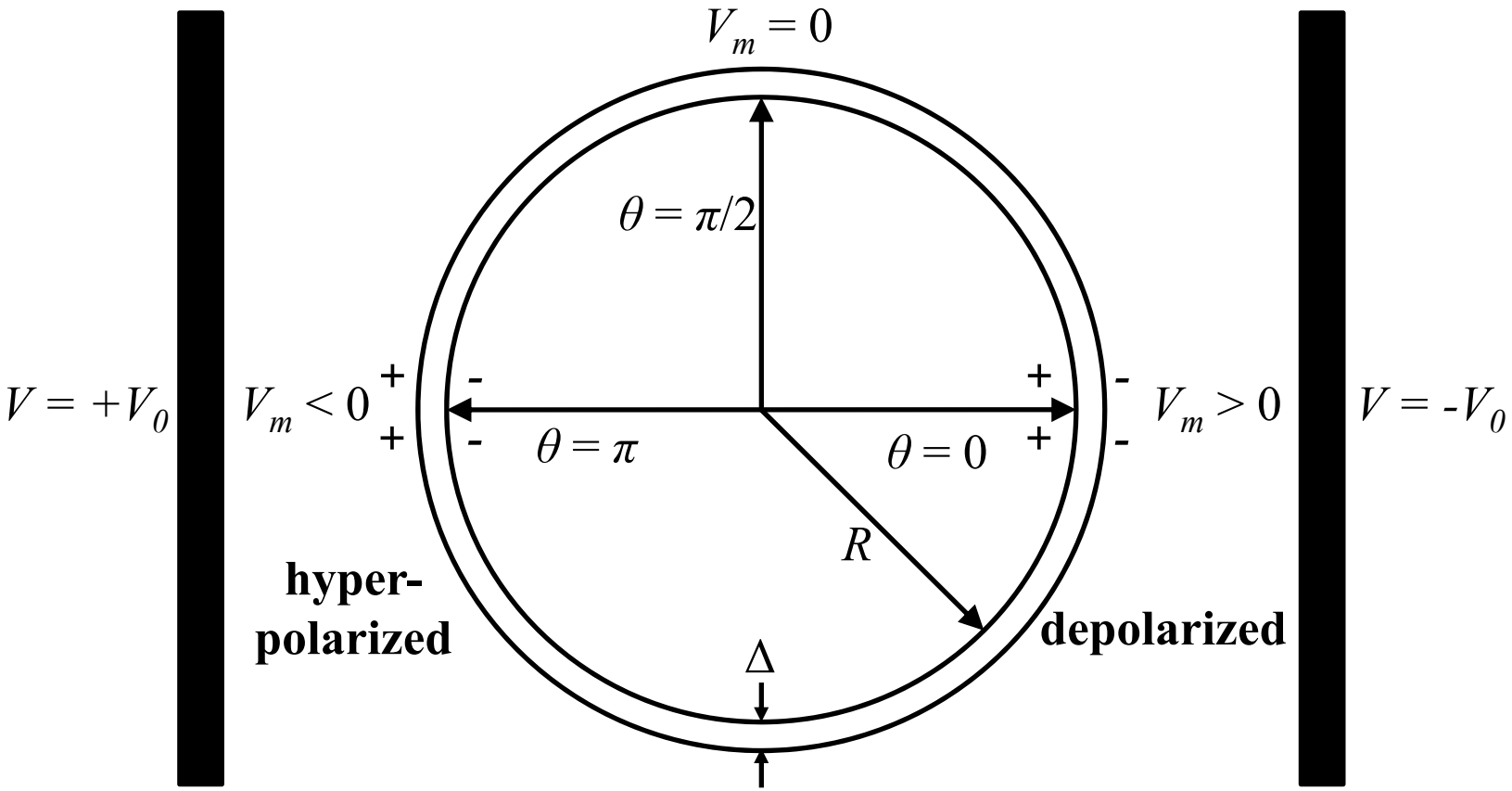
$$V_{dipole} = \frac{p \cdot \cos\theta}{4 \cdot \pi \cdot \sigma \cdot r^2}$$

MODEL OF ELECTRIC STIMULATION





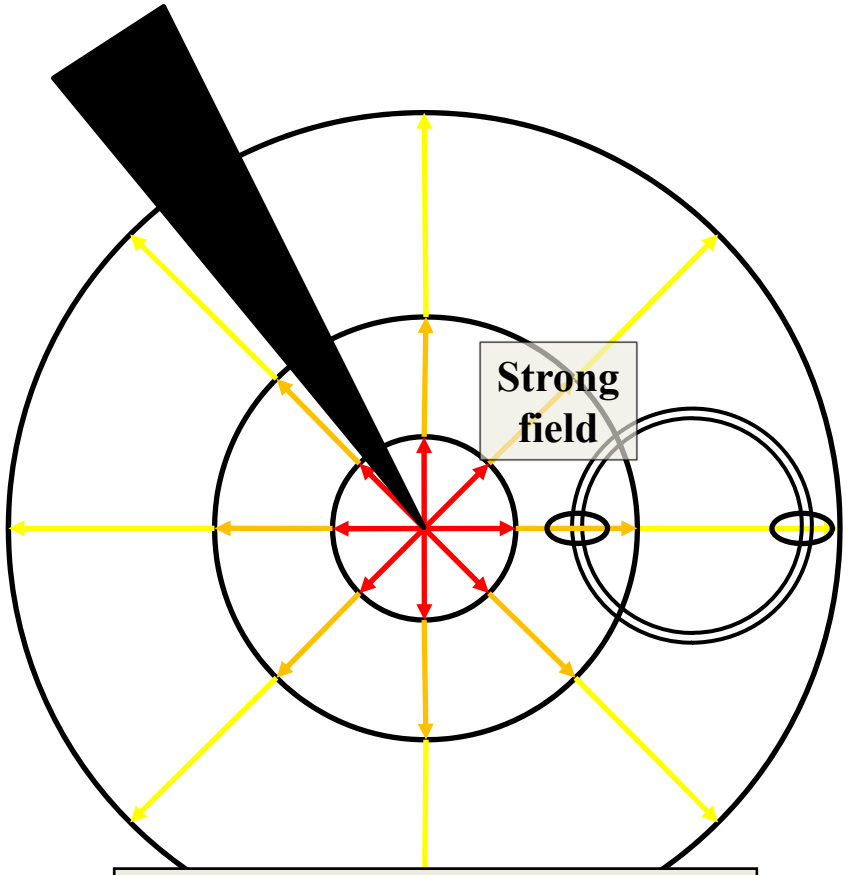




$$V_m = V_{r=R} - V_{r=R+\Delta}$$

$$V_m \sim E_0 \cdot \cos(\theta)$$

$$\theta = 0 \rightarrow Vm > 0 \rightarrow \textit{depolarized}$$
$$\theta = \pi/2 \rightarrow Vm = 0 \rightarrow \textit{depolarized}$$
$$\theta = \pi \rightarrow Vm < 0 \rightarrow \textit{depolarized}$$



$$J(R) = \frac{I}{A(R)} = \frac{I}{4 \cdot \pi \cdot r^2}$$

Electrical stimulation means a functional change in a nerve (muscle) cell due to electric current.

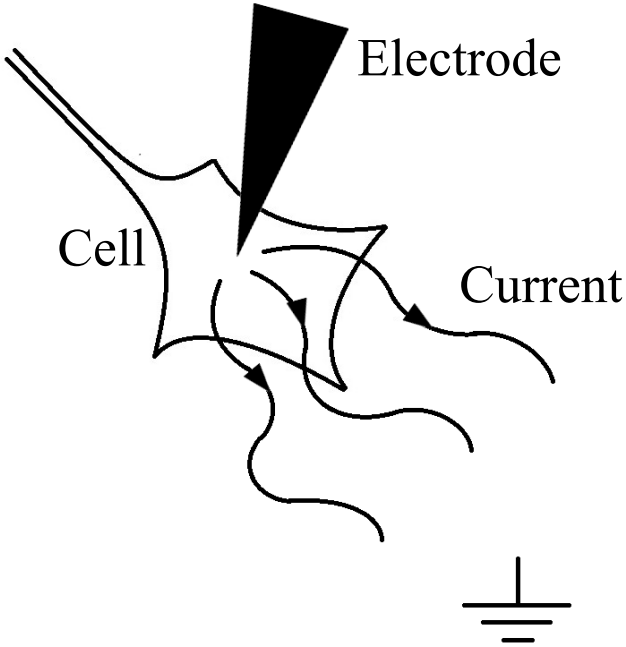
Needed:

- Excitable cell
- Electric current
- **Stimulating electrode**

Stimulating electrode:

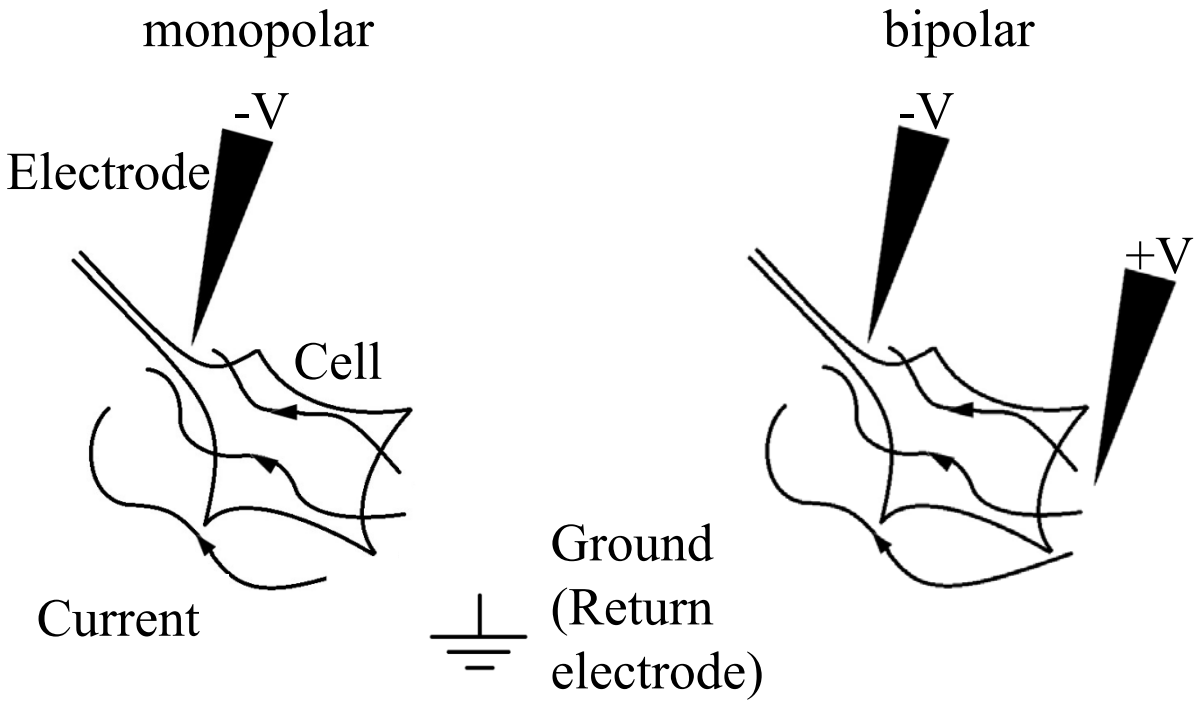
- Types of stimulation
- Electrode properties
- Stimulation effects

INTRACELLULAR STIMULATION WITH ELECTRIC CURRENT



Direct control over all ion currents.
Technical implemetation is hard.

EXTRACELLULAR STIMULATION WITH ELECTRIC CURRENT

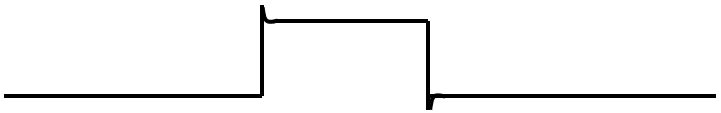


Indirect control over all ion currents.
Technical implemetation is easier.

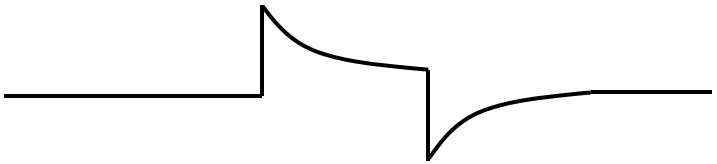
CHARACTERISTICS OF VARIOUS ELECTRODES

Current

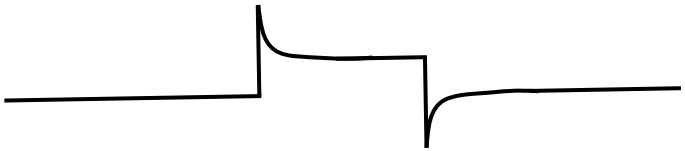
Ag/AgCl



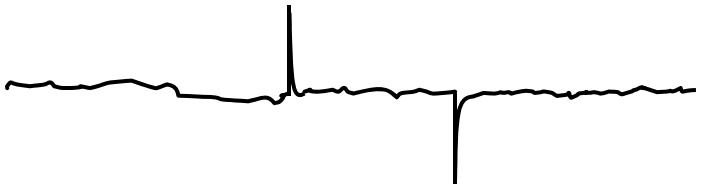
Platinum



Copper

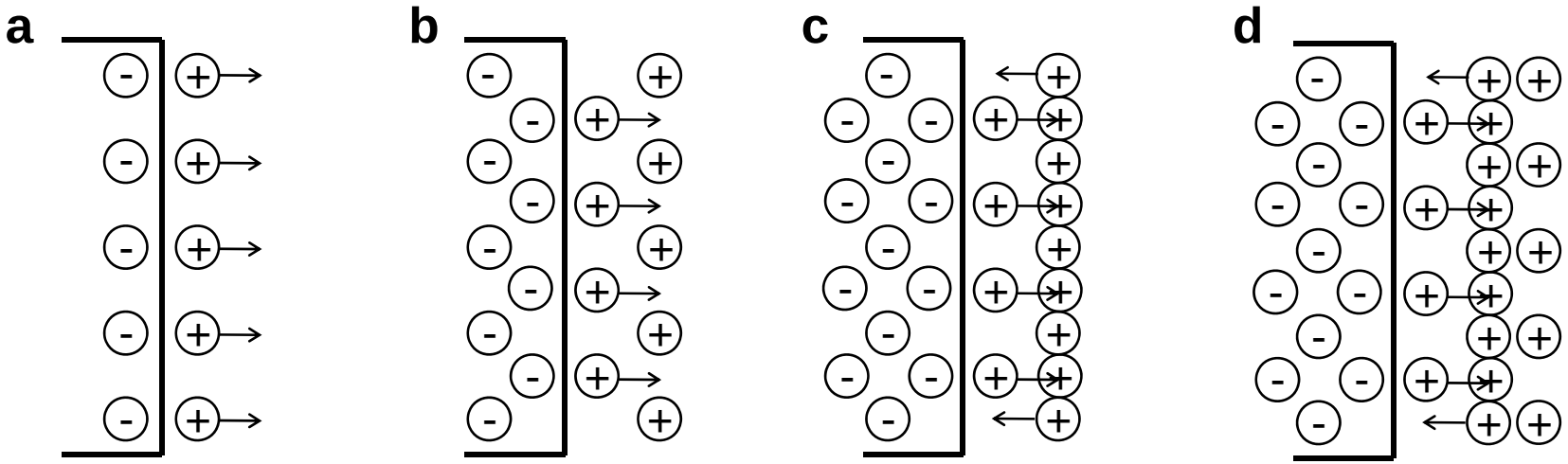


Stainless steel



ELECTRICAL DOUBLE LAYER

Ion movement during metal-liquid contact → polarization → equilibrium



(a) Ionic flow into solution immediately after immersion,
(b) accumulation of ions in solution,
(c) ionic flow into and out of solution at different rates,
(d) equilibrium when rates are equal.

(Cooper, 1962)

POLARIZABLE ELECTRODES

Metals: Ag, Pt, Au, etc.

Polarizable electrodes:

- amount of current depends on properties of double layer (C, R)
- current charges or discharges the double layer
- current vs. voltage is non-linear
- DC impedance is big, acts as capacitance

but can be decreased by increasing the surface.

NON-POLARIZABLE ELECTRODES

Ag/AgCl

Non-polarizable electrodes:

- current does not influence properties of double layer
- current flows freely on the double layer
- current vs. voltage is linear
- acts as resistance on DC

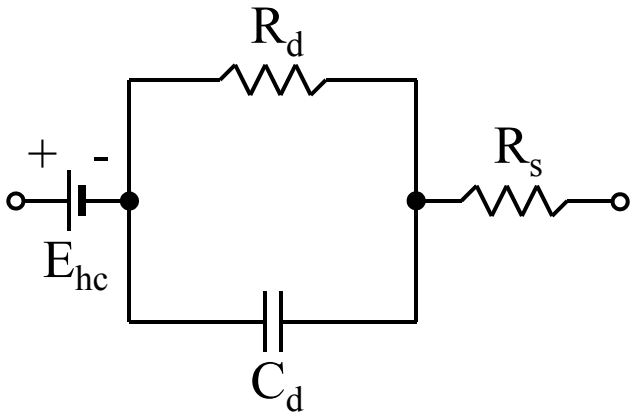
GALVANIC SERIES

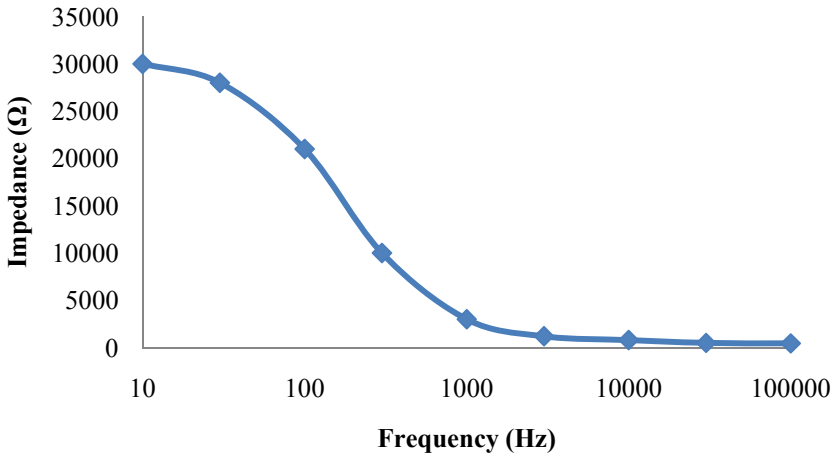
In case of metal-liquid-metal contact they form a galvanic cell.
Electrode potential is the potential difference relative to the standard hydrogen electrode.
Metals with positive potential to hydrogen are called noble metals.

Metals more reactive than hydrogen	Potassium	K
	Sodium	Na
	Calcium	Ca
	Magnesium	Mg
	Aluminium	Al
	Zinc	Zn
	Iron	Fe
	Hydrogen	**H**
Metals less reactive than hydrogen	Copper	Cu
	Silver	Ag
	Platinum	Pt
	Gold	Au

Decreasing chemical reactivity

MODEL OF ELECTRODE-ELECTROLYTE INTERFACE





E_{hc}: half-cell potential

R_s: electrolyte resistance

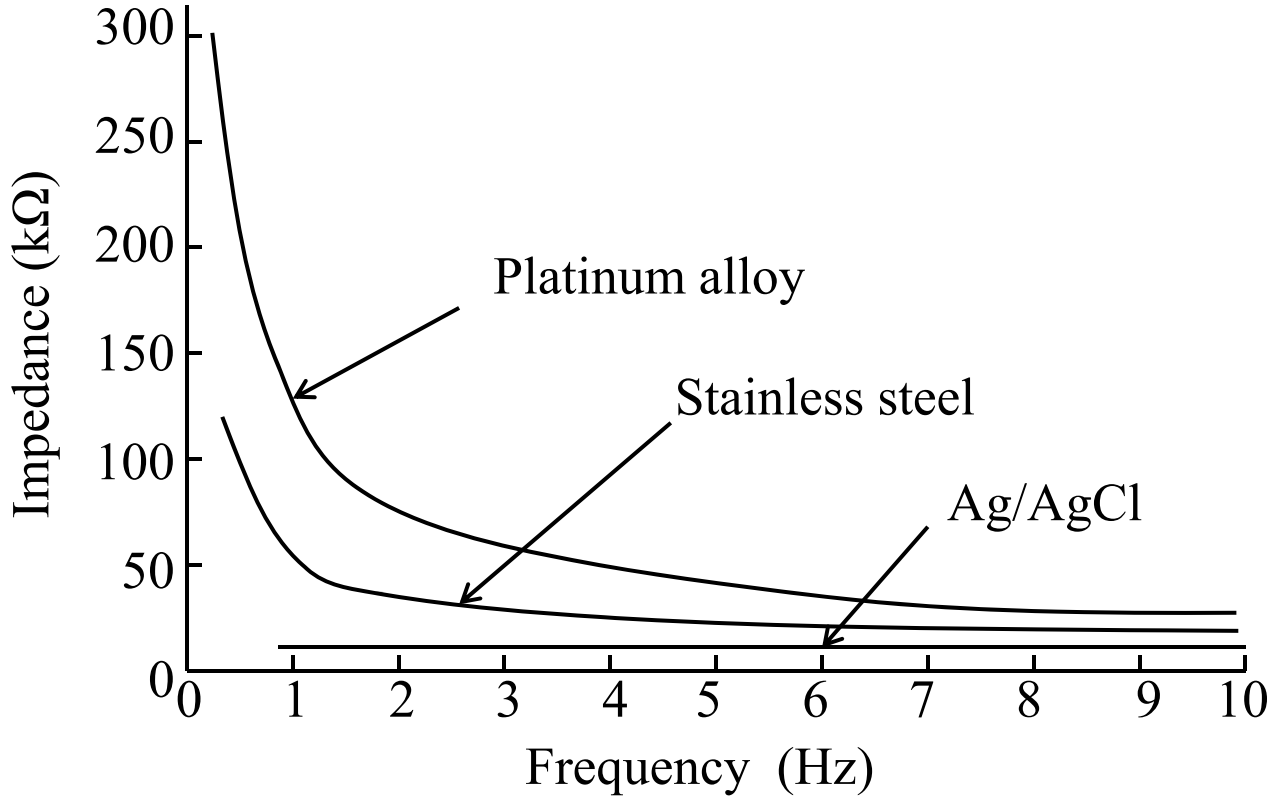
R_d: interface resistance

$$Z_C = R_s + \frac{R_d \frac{1}{j \cdot \omega \cdot C}}{R_d + \frac{1}{j \cdot \omega \cdot C}}$$

Z_C: electrode impedance

C: capacitance

FREQUENCY-DEPENDENCE OF ELECTRODE IMPEDANCE



STIMULATION WITH REPETITIVE IMPULSES

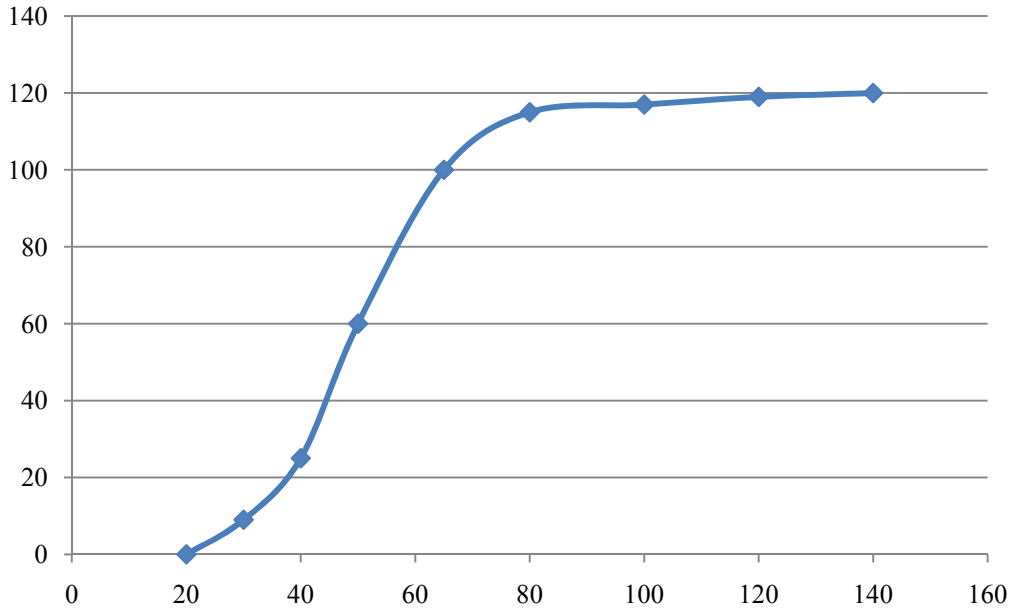
High current density → Hydrolysis, Thermic effect, Charge injection → Tissue damage

Monophasic repetitive stimulation

Biphasic repetitive stimulation

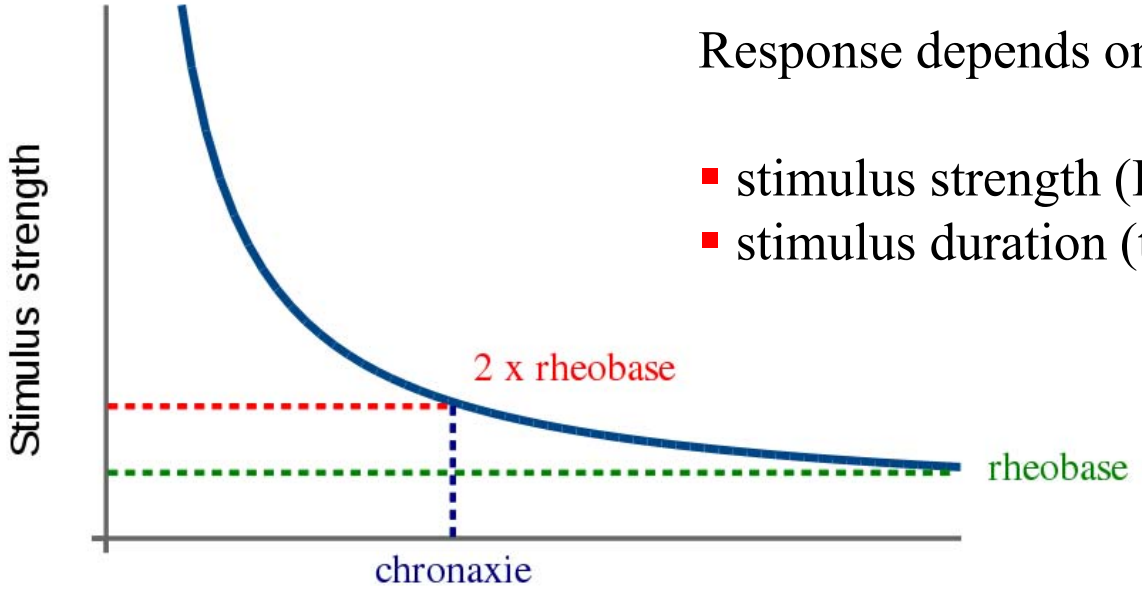
STIMULUS VS. RESPONSE

Muscle contraction torque (ft*lbs)



Stimulus intensity (mA)

STIMULUS STRENGTH-DURATION CURVE



Response depends on:

- stimulus strength (Is)
- stimulus duration (t)

$$I_s = \frac{I_{rh}}{1 - e^{-t/\tau}}$$

Rheobase (Irh): the minimal current amplitude of infinite duration that results in an action potential.

Chronaxy (Chr): the minimum time over which an electric current double the strength of the rheobase needs to be applied, in order to generate an action potential.

SUMMARY

- ionselective (semipermeable) membrane
- membrane permeable for water, not permeable for ions
- condition for excitation and inhibition: ion concentration difference between the two sides of the membrane
- voltage on membrane depends on diffusion and electrostatic forces
- in Nernst equilibrium the voltage is proportional to the logarithm of the quotient of concentrations
- in Nernst equilibrium there is no net current flow on ion channels
- resting potential is determined by ion concentration differences and ion channel permeabilities
- the most important mobile ions are Na, K, and Cl
- intracellularly many negatively charged proteins and K, extracellularly many Na and Cl ions
- $P_K > P_{Cl} > P_{Na}$

SUMMARY

- ions in dynamic balance, no real equilibrium, leaky channels
- leak compensated by energy demanding pumps (Na-K pump)
- Goldman equation gives good approximation for resting potential
- depolarization $V_m > V_r$, hyperpolarization $V_m < V_r$
- increasing permeability of ion channel pushes resting potential towards equilibrium (Nernst) potential of given ion
- increasing P_{Na} depolarizes (inward Na flow), increasing P_K hyperpolarizes (outward K flow)
- increasing P_{Cl} may either depolarize or hyperpolarize, depending on equilibrium potential of Cl

SUMMARY

- Poisson equation describes the relationship between potential and current source density.
- Electric field of monopole, dipole
- Monopolar, bipolar stimulation
- Formation of electrical double layer
- Polarizable, non-polarizable electrodes
- Monophasic, biphasic stimulation
- Rheobase is the minimal current amplitude of infinite duration that results in an action potential.
- Chronaxy is the minimum time over which an electric current double the strength of the rheobase needs to be applied, in order to generate an action potential.

REFERENCES

- Kandel, E.R., Schwartz, J.H., Jessel, T.M.: Principles of Neural Science, 4th ed., McGraw-Hill, New York, 2000.
- Squire, L.R., Bloom, F.E., McConnell, S.K., Roberts, J.L., Spitzer, N.C., Zigmond, M.J.: Fundamental Neuroscience, 2nd. ed. Academic Press, 2003.
- MalmivuoJ., Plonsey, R.: Bioelectromagnetism, *http://www.bem.fi/book/index.htm 1995.*

EXTERNAL LINKS

- http://www.youtube.com/watch?v=DF04XPBj5uc
- http://www.youtube.com/watch?v=1ZFqOvxXg9M
- http://www.youtube.com/watch?v=owEgqrq51zY
- http://www.youtube.com/watch?v=s0p1ztrbXPY
- http://bcs.whfreeman.com/thelifewire/content/chp44/4402001.html

REVIEW QUESTIONS

- What is the structure of a neuron membrane?
- What types of ion channels do you know?
- What forces drive the ions through the membrane?
- What is the Nernst equilibrium?
- What does the Goldman-Hodgkin-Katz equation tell?
- What is electric stimulation?
- What are the building blocks of the neuron membrane?
- How does a stimulating electric current change the membrane potential?
- What does the Poisson equation tell?
- What is the electric field of a monopole/dipole?
- What is the difference between intracellular and extracellular stimulation?
- What are the properties of the double layer?
- What is the difference between polarizable and non-polarizable electrodes?
- Tell some examples of non-polarizable electrodes!
- What is rheobase?
- What is chronaxy?